Three-dimensional Ultrasound Imaging of Regenerated Skin with High Frequency Ultrasound

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***Abstract*—Regenerated skin with complete organ structure is desired for the treatment of large burn and for the plastic surgery. High frequency ultrasound is suitable for non-destructive testing of such skin models because it can obtain both morphological and biomechanical information in non-invasive manner. A specially developed acoustic microscope system with the central frequency of 100 MHz was developed for evaluation of regenerated skin. The system was capable of (1) conventional C-mode acoustic microscope imaging of thinly sliced tissue, (2) ultrasound impedance *in vivo* imaging of the surface of tissue and (3) 3D ultrasound imaging of *in vivo* tissue. Sound speed in C-mode showed higher values at the area of dense fibroblasts in collagen sponge. The system can be used as the *in vitro* nondestructive evaluation tool in the process of regenerating skin and as the *in vivo* diagnostic system after implantation of the skin.**

Keywords-component; acoustic microscopy; skin; signal processing; speed measurement

I. INTRODUCTION

A. Current Status of Regenerated Skin

Three-dimensionally regenerated skin with epidermis, dermis, hair follicle, lipid gland and capillary is desired for the treatment of large burn or for plastic surgery. Not only the structure, should the skin model be flexible as normal skin.

Artificial dermis, Pelnac® (Gunze, Kyoto, Japan) was commercialized in Japan in 1996 [1]. Pelnac consisted of collagen sponge covered with silicon film. As it needed autologous epidermis coverage, repeated operation was required.

Cultured epidermis was approved in Japan on October 3, 2007 for orphan use of severe skin burn. Skin biopsy from the patient is performed to obtain autologous epidermis and keratinocytes are isolated from the tissue. Cells are cultured to form sheet structure and finally the sheeted epidermis is transplanted to the patient. As the epidermis is autologous, risk of rejection is avoided [2]. However, the cultured epidermis is only grown in the skin with dermis. Then it cannot be used in the case of severe burn without dermis.

Cultured dermis has the same structure as artificial dermis. Fibroblasts are cultured in the collagen sponge layer of the artificial dermis. The model was slightly advanced from the artificial dermis but it still needed autologous epidermis coverage.

Apligraf® (Organogenesis Inc., Canton, MA, USA) is bi-layered skin substitute consisting of living cells and structural proteins. The lower dermal layer combines bovine type 1 collagen and human fibroblasts (dermal cells), which produce additional matrix proteins. The upper epidermal layer is formed by promoting human keratinocytes (epidermal cells) first to multiply and then to differentiate to replicate the architecture of the human epidermis [3].

Another cultured 3D skin model, Vitrolife-Skin® (Gunze, Kyoto, Japan) is 3D human skin model used as an alternative for animal skin during irritation test. Keratinocytes are cultured to cover cultured epidermis to form two-layered structure [4].

Fig. 1 shows the schematic illustration of each regenerated skin model.

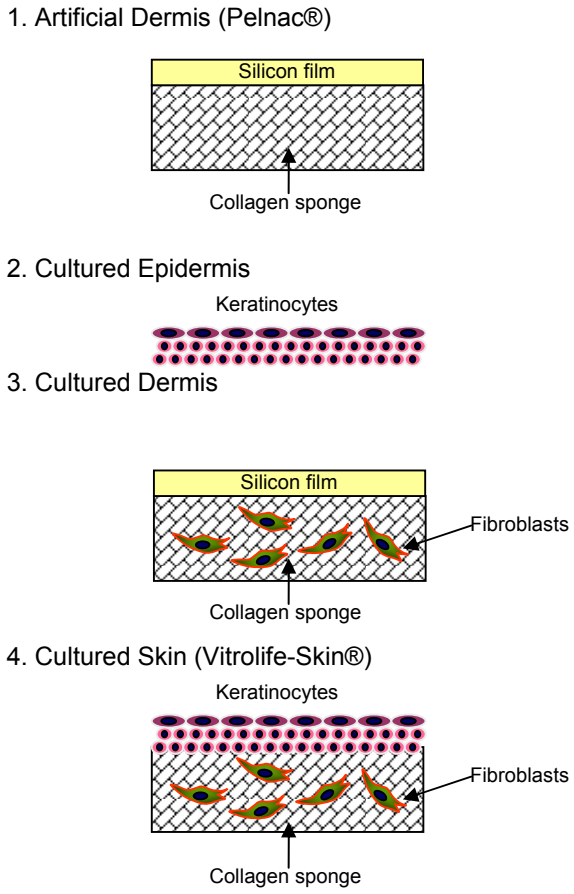


Fig. 1 Schematic illustrations of regenerated skin models

Although these cultured skins have bi-layered structure, they do not contain melanocytes, Langerhans' cells, macrophages, and lymphocytes, or other structures such as blood vessels, hair follicles or sweat glands. As they don't have vasculature, barrier function against bacterial infection is very weak. Their elastic property is different from normal skin because they are lacking elastic fiber.

B. Three-dimensional Complex Organ Structures

The three-dimensional molding methods and noninvasive evaluation techniques are fully employed to realize structures that are morphologically and functionally similar to those of a living body. This will enable the realization of large-size structures, regeneration of organ structures suitable to anatomical morphology, and functional reconstruction by applying various engineering techniques that are difficult to produce with present tissue engineering. At the same time, revascularization in the host site, which is necessary for achieving graft adhesion and self-organization of the three-dimensional complex organ structures, will be realized.

As a part of this research project, 3D skin model with bi-layer structure, elastic fiber and vasculature is aimed and non-invasive repetitive evaluation method is desired. High frequency ultrasound is suitable for non-destructive testing of the skin model because it provides information on morphology and mechanical properties. In the present study, the same plane, which was perpendicular to the skin surface, of the skin model with 3D structure was investigated with both B-mode and C-mode ultrasound imaging using 100 MHz ultrasound.

II. METHOD

A. System Setup

Fig. 2 shows the schematic illustration of the B-mode and C-mode imaging system. An electric impulse was generated by a high speed switching semiconductor. The start of the pulse was within 400 ps, the pulse width was 2 ns, and the pulse voltage was 40 V. The frequency of the impulse covered up to 500 MHz. The electric pulse was used to excite a PVDF transducer with the central frequency of 100 MHz. The ultrasound spectrum of the reflected ultrasound was broad enough to cover 50-160 MHz (-6dB). The reflections from the tissue was received by the transducer and were introduced into a Windows-based PC (Pentium D, 3.0 GHz, 2GB RAM, 250GB HDD) via a high-speed A/D converter (Acqiris DP210, Geneva, Switzerland). The frequency range was 500 MHz, and the sampling rate was 2 GS/s. Eight pulse echo sequences were averaged for each scan point in order to increase the signal-to-noise-ratio. The transducer was mounted on an X-Y stage with a microcomputer board that was driven by the PC through RS232C. The Both X-scan and Y-scan were driven by linear servo motors.

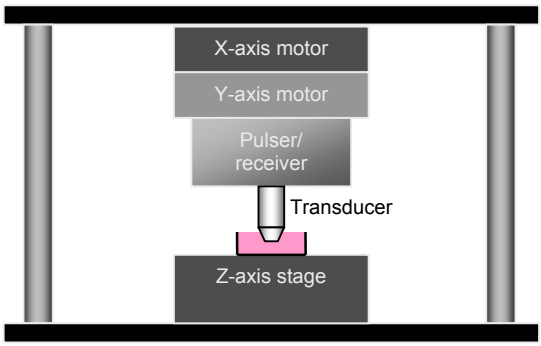


Fig. 2 Schematic illustrations of the B-mode and C-mode imaging system

B. Ultrasonic Transducers

B-mode imaging was obtained with a PVDF transducer with the diameter of 2.4 mm and the focal length of 3.2 mm. C-mode imaging was obtained with a different PVDF transducer with the diameter of 1.8 mm and the focal length of 1.5 mm.

C. Signal Processing

RF signal of each scanning line was converted to B-mode image by a conventional image processing algorithm. The scan area was 2.4 mm x 3.0 mm with 300 x 4000 pixels. Y scan width was available 8 / 16 / 24 / 64 microns step to obtain 3D data set.

The transfer function of the pulsed response at tissue region and glass region was calculated in a frequency domain to

calculate the tissue thickness, attenuation and sound speed in C-mode imaging [5-7].

D. Tissue Preparation

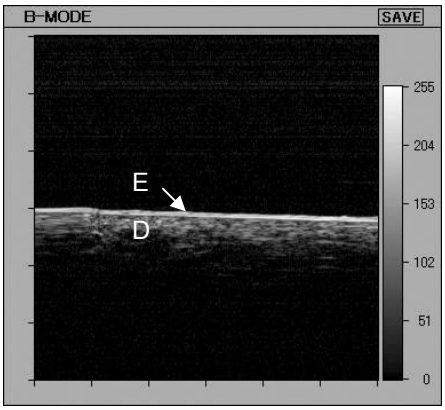
Fibroblasts in Vitrolife-Skin were cultured with the Dulbecco's modified Eagle's medium and 10% heat-incubated bovine serum. The incubator was maintained at 37 °C and filled with 95% air and 5% CO_2.

First, B-mode images were obtained by using the saline as the coupling medium. After B-mode evaluation, the sample was frozen and sliced at 5 micron in thickness as to make the same observation plane for C-mode imaging. The neighboring sections of C-mode image were stained with Elastica-Masson staining for optical microscopic observation.

III. RESULTS

Fig. 3 shows (a) B-mode and (b) 3D ultrasound images of Vitrolife-Skin. Epidermis (E) was observed as a relatively high echoic band in the B-mode imaging. Dermis (D) consisted of collagen sponge and fibroblasts had lower echo intensity. 3D ultrasound image showed clear surface morphology.

(a)



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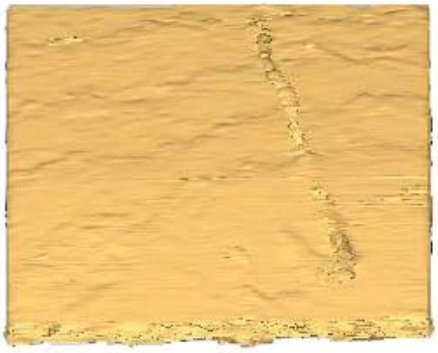
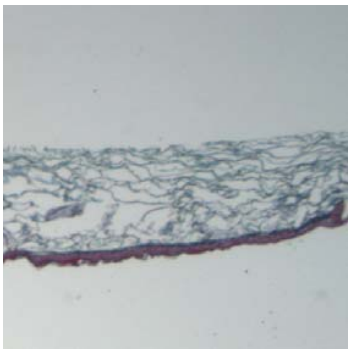


Fig. 3 (a) B-mode and (b) 3D ultrasound images of Vitrolife-Skin

Fig. 4 shows the (a) optical microscopic and C-mode images ((b): attenuation, (c): sound speed) of Vitrolife-Skin. The sound speed of the epidermis was approximately 1580 m/sand the sound speed of dermis was ranged 1530 to 1560 m/s corresponding to the density of fibroblasts in the dermis.

(a)



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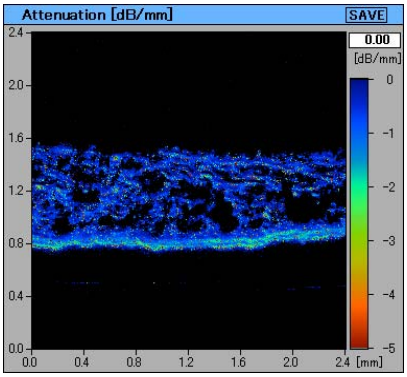

(c)

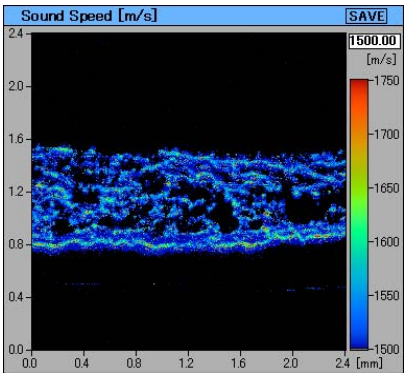

Fig. 4 (a) optical microscopic and C-mode ((b): attenuation, (c): sound speed) images of Vitrolife-Skin

The density of fibroblast (number / 0.2x0.2 mm) and the sound speed of the corresponding region were measured in 12 regions from 6 specimens. Fig. 5 shows the relationship between tow parameters. The result suggests there is a strong relationship between cellularities and sound speed.

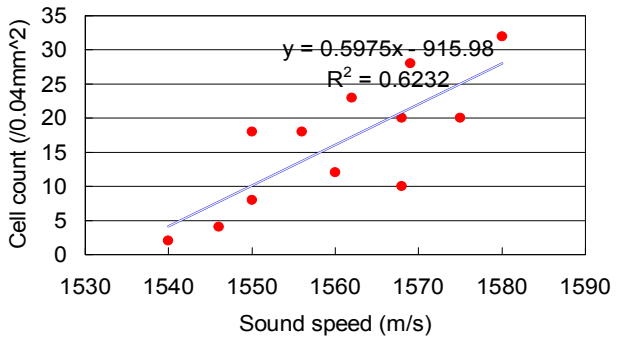

Fig. 5 Relationship between cell count and sound speed

IV. Discussion

Compared with conventional high frequency B-mode imaging modalities, this system is unique because it can be used as C-mode acoustic microscopy. C-mode quantitative values are important for understanding *in vivo* B-mode images. As the spatial resolution of the system is 15 micron, it is enough to visualize epidermis with 50-100 micron thickness. However, dermis consisting of collagen sponge and fibroblasts was hard to visualize in case the cellularties was very sparse.

One of the future directions of this imaging modality is nondestructive testing of the regenerated skin during tissue culture because it can clearly visualize the cellular density in the dermis and because non-invasive, non-contact, non-infectious method is desired for evaluation of skins. It also has a promising future as a medical imaging device. Continuous evaluation during culture and after transplantation can be performed by the same imaging system in the regenerative medicine. It can be used as a diagnostic device in dermatology. As the system clearly shows the thickness of epidermis and mechanical properties represented acoustic parameters, the system is applicable in cosmetic care of skin.

V. Conclusion

Regenerated skin with complete organ structure is desired for the treatment of large burn and for the plastic surgery. High frequency ultrasound is suitable for non-destructive testing of such skin models because it can obtain both morphological and biomechanical information in non-invasive manner. A specially developed acoustic microscope system with the central frequency of 100 MHz was developed for evaluation of regenerated skin. The system was capable of (1) conventional C-mode acoustic microscope imaging of thinly sliced tissue, (2) ultrasound impedance in vivo imaging of the surface of tissue and (3) 3D ultrasound imaging of in vivo tissue. Sound speed in C-mode showed higher values at the area of dense fibroblasts in collagen sponge. The system can be used as the in vitro nondestructive evaluation tool in the process of regenerating skin and as the in vivo diagnostic system after implantation of the skin.

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